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# Amphibian conservation through captive breeding: the case of the harlequin toads (Anura, Bufonidae, *Atelopus*)

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## Abstract

Amphibians are the most threatened class of terrestrial vertebrates: approximately 41% of species are on the brink of extinction or in decline due to habitat loss, climate change, and emerging diseases. Projects involving ex situ and in situ conservation are crucial to preserving the integrity and future of many amphibian populations and species. This paper reviews some of the conservation initiatives developed for species in the genus *Atelopus* (Amphibia, Bufonidae). It also focuses particularly on ex situ conservation activities carried out for *A. balios*, providing an overview of the results.

**Keywords**. Amphibian decline, *Atelopus*, harlequin toads, ex situ conservation projects.

## Riassunto

Gli anfibi sono attualmente la classe di vertebrati terrestri più in pericolo. Circa il 41% delle specie è sull'orlo dell'estinzione o in forte declino a causa della perdita e dell'alterazione degli habitat, del cambiamento climatico e di patologie emergenti. Pertanto, i progetti di conservazione ex situ e in situ sono di fondamentale importanza per la preservazione dell'integrità e il futuro delle popolazioni di molte specie. Le strategie finora adottate sono varie e in questa review discutiamo di alcune delle modalità ed iniziative di conservazione tramite allevamento in cattività, adottate per le specie di *Atelopus* (Amphibia, Bufonidae) a rischio. In particolare, questo lavoro fornisce anche una sintesi delle attività di conservazione ex situ messe in atto per *A. balios*.

**Parole chiave**. Declino globale degli anfibi, *Atelopus*, rospi arlecchino, progetti di conservazione ex situ

## Introduction

According to the Second Global Amphibian Assessment, around 41% of amphibian species worldwide are currently threatened with extinction (Luetdke et al., 2023). Indeed, amphibians are one of the vertebrate groups most affected by this decline and are considered a key indicator of the ongoing global biodiversity crisis. Major threats include habitat destruction and fragmentation caused by human activities, pollution from agricultural and industrial sources and the rapid spread of diseases such as chytridiomycosis, which is caused by the fungal pathogens, such as *Batrachochytrium dendrobatidis* (*Bd*) and *B. salamandrivorans* (*Bsal*) (Luetdke et al., 2023). These pressures have had a drastic effect on populations, resulting in local extinctions and severe disruption to ecosystems.

In this context, species belonging to the *Atelopus* genus (commonly known as "harlequin toads") were particularly affected: many *Atelopus* populations have suffered sharp declines in recent decades, with several species currently classified as Critically Endangered (CR) or Extinct in the Wild (EW) (Lötters et al., 2023). So far, it is clear that conservation efforts targeting these species therefore require urgent integration of in situ and ex situ strategies.

The current review aims to explore some of the key risks and threats affecting *Atelopus* species, and to provide a synthesis of the conservation measures and initiatives implemented at local and international levels. Emphasis has been given on ex situ conservation programmes, which have emerged as a crucial tool for preventing species extinction, particularly when natural populations can no longer be protected in their native habitats (Pavajeau et al., 2008). Particular attention has been given to actions involving captive breeding techniques developed for *A. balios*, shedding light on the challenges, advances and prospects of these approaches.

## The harlequin frogs

The genus *Atelopus* is included within the Bufonidae family (true toads), despite their overall "frog-like" appearance. Their main morphological traits have often misled biologists regarding their phylogeny, since features such as brilliant skin colouration (the origin of the name "harlequin frogs") (fig. 1) and the presence of skin-produced toxins are widespread in several anuran families (e.g. Dendrobatidae, Mantellidae, Microhylidae and Phyllomedusidae). The 105 *Atelopus* species (at the 14 December 2025, https://amphibiaweb.org/) are characterised morphologically by small-to-medium body sizes (17–50 mm), smooth dorsal skin, a conspicuous absence of parotoid glands, well-developed interdigital webbing, the reduction of the first digit, lack of the middle ear in most of the species and vivid aposematic colouration correlated with the presence of skin alkaloids and bufadienolide toxins used for chemical defence (Lötters et al., 2011). The species are distributed in the forests of Central America (Panama) and South America (Colombia, Ecuador and Venezuela) (Amphibiaweb, 2025). The last IUCN report assessed 94 species of *Atelopus*, classifying 62 as Critically Endangered (CR), with 39 of these possibly already Extinct in the Wild (EW) (Lötters et al., 2023).

### Threats affecting the harlequin toads

Anthropogenic activities often have a negative impact on global biodiversity, leading to habitat degradation and fragmentation, as well as the spread of emerging diseases

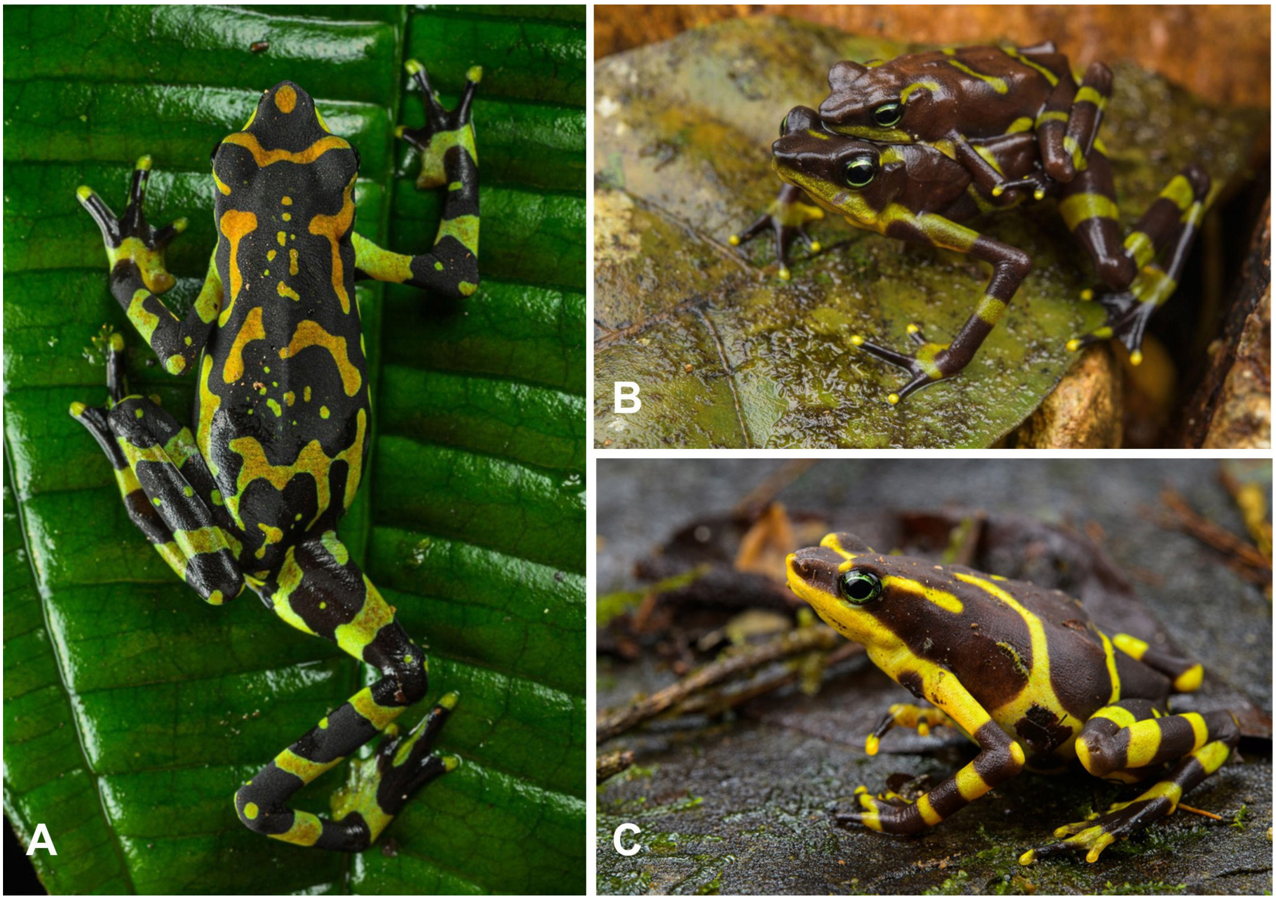


**Figure 1**: Variability within the genus *Atelopus*: (A) *Atelopus varius* "morph C", (B) *Atelopus limosus*, (C) *Atelopus glyphus.* (© Emanuele Biggi)

(Suriyamongkol et al., 2021; Rosas-Espinoza et al., 2022; Yu et al., 2022). These effects are particularly pronounced in sensitive organisms, such as amphibians, which are closely associated with humid environments and often lead double lives as aquatic tadpoles and terrestrial adults. According to the 2023 IUCN Red List, 91% of species belonging to the orders Anura, Urodela and Gymnophiona experienced a significant decline in their conservation status between 1980 and 2004. One of the most pressing threats is the spread of emerging diseases, such as chytridiomycosis, which is caused by two species of *Batrachochytrium: B. dendrobatidis and B. salamandrivorans* (Fisher et al., 2009), which are aquatic fungi belonging to the order Chytridiales. Both *Batrachochytrium* species likely originated in Asia and have since spread worldwide through human activities (Martel et al., 2014). These fungi have contributed to the decline of over 500 amphibian species, accounting for at least 6.5% of all described amphibian species (Scheele et al., 2019). Twenty-seven *Atelopus* species declined rapidly from 1984 to 2004; more than half of these had not been seen for a long period of time, and only 10 had stable populations.

## Ex situ conservation: goals and opportunities

Ex situ conservation provides a vital safety net and a powerful tool for research, especially when in situ efforts are compromised or the species is difficult to study in its natural habitat. Ex situ conservation programs have been successfully conducted for several threatened amphibian species (i.e, Harris et al., 2022).

A major problem with studies focused on threatened amphibians is sampling. The elusive nature of some species, coupled with the loss of many ecological niches, can mislead or even prevent the collection of new data on presumed threatened species. Harlequin toads are a prime example of this.

Studies based on pathogen-induced mortality, infection rates and population recruitment rates demonstrate that these species have coexisted in endemic equilibrium with Bd for almost a decade (Ballestas et al., 2021). This highlights the importance of in situ research to improve our understanding of trends in anuran populations.

Captive breeding programmes are fundamental to establishing ex situ populations, particularly for highly endemic anurans that are threatened by diseases and human activities. In some cases, these programmes represent "the last hope" of saving the species from extinction. It is also important to note that these strategies do not offer a permanent solution to the problem, but rather a plan for the recovery of wild populations (Gascon et al., 2005).

These projects are a useful tool for extending the time available to mitigate wild threats and provide a viable source of animals for reintroduction into their native habitats. Many organisations contribute to this effort, primarily universities and zoos, creating a large network with a main objective: preserve species by rearing and breeding them in specialised facilities and finally reintroducing them into the wild. Eighty-four or more species of animals and plants are classified as EW by the IUCN (Dalrymple et al., 2023), suggesting that wild populations no longer exist and are represented only by captive nuclei. Furthermore, ex situ strategies sometimes have good success rates, but there are still some risks that could jeopardise the project's positive outcome.

Genetic stochasticity, particularly in amphibians with small populations, that are exposed to the risk of diffusing new pathogens in local environments where threatened species breeding centres are located, potentially represents one of the major problems of ex situ strategies. However, advances in biosecurity and safety protocols nowadays help to mitigate these risks by emphasising the maintenance of native species within dedicated facilities and, in some cases, by restricting the introduction of non-native taxa. While these measures are not directly linked to the pet trade, they reflect standard biosecurity practices aimed at preventing pathogen spillover and safeguarding local biodiversity (Zippel et al., 2011).

In terms of threatened amphibian conservation, we mention the "El Valle Amphibian Conservation Centre", located in the city of El Valle de Antón in Panama (Kolbert, 2016). This facility is dedicated to conserving several *Atelopus* species, including the Panamanian golden frog (*Atelopus zeteki*) (fig.2). The centre is part of the wider Panama Amphibian Rescue and Conservation Project. Another important centre is the "Centro Jambatu" in Ecuador, where field biologists, conservationists, educators and communicators meet to focus on the conservation of several species (http://www.anfibiosecuador.ec/). This breeding center has the world's largest and most valuable collection of *Atelopus*, with fourteen live species and twenty-eight other species of frogs, toads, and urodeles. The center is fo-

**Figure 2**: *Atelopus zeteki*. (© Emanuele Biggi)

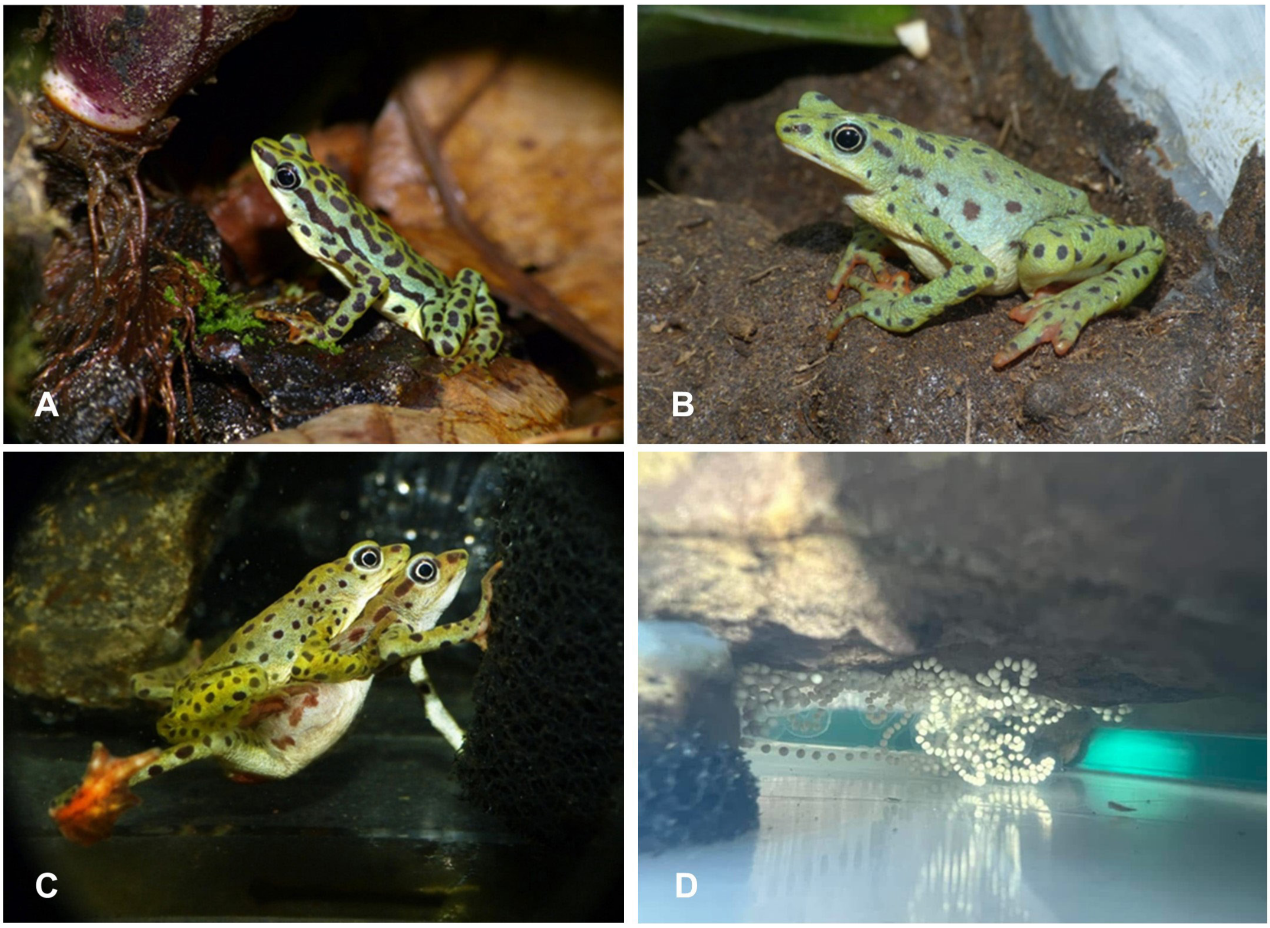


**Figure 3**: *Atelopus balios*. A) male; B) female. C) Amplexus D) Egg clutch. (©Thomas Ackermann)

cused not only on preserving live threatened animals, but also on preserving specimens for research and conservation purposes.

It is also crucial to specify when and how an amphibian species should be included in an ex situ conservation programme. Planning is one of the most important phases of a conservation effort, and its structure varies depending on which species deserves the attention of scientists and the available economic resources. Many amphibian taxa are featured by reproductive strategies involving many eggs and no parental care, making them suitable for short and long-term conservation activities through captivity (Griffiths & Pavajeau, 2008). However, difficulties still exist regarding the training and monitoring of reintroduced individuals (Bloxam et al., 1995).

### The Citizen Conservation project for *Atelopus balios*

The Citizen Conservation (CC) is a programme developed in 2018 by the German organisation Frogs & Friends (https://citizen-conservation.org/), with the aim to include private animal breeders in ex situ conservation projects, producing a connection between institutions and hobbyists and having a positive impact on many threatened species. CC currently has an Italian chapter, which is managed by the Italian Gekko Association (Di Martino & Carsana, 2025).

CC's partners actually raise twenty species of frogs, toads and fish. Here, we provide some information on CC's initiatives concerning *Atelopus balios*, also known as the Rio Pescado stub-foot toad (Figs. 3A-B). This anuran

was once widespread on the riverbanks and in the lowland valleys of southwestern Ecuador (Peters, 1973). *Atelopus balios* is recognisable by its light green livery with black spots on the back and the characteristic orange toes found in many Anura taxa (Monnet et al., 2002; Olivera-López et al., 2021). Females are larger than males, with a snout-to-vent length (SVL) of 40–50 mm in females and 30–38 mm in males (Fig. 3A, B) (Buttermore et al., 2024).
*Atelopus balios* was thought to be extinct, but it was rediscovered in 2010, fifteen years after it was last seen in 1995 (Hance, 2011). Since then, it has been included in many projects aimed at saving it from extinction. CC is one of these projects, offering its support for the ex situ conservation programme, including captive husbandry, alongside other zoos and aquaria.

## Breeding, rearing tadpoles and toadlets

Similarly to other harlequin toads, *Atelopus balios* has been bred in captivity by the Centro Jambatu and Amaru Bioparque in Cuenca and is currently included in the CC's conservation activities. The Rio Pescado Stubfoot Toad breeding programme involves keeping diverse individuals in a controlled environment using naturalistic-style terrariums, or keeping the animals in a simpler, more hygienic set-up . The former method is favoured by private keepers, primarily for aesthetic reasons. The overall size of the terrariums is directly proportional to the number of toads. This is due because *Atelopus* usually feeds on large quantities of small insects: providing substantial space for a small group of animals makes this behaviour quite challenging. Temperatures should be 22-25 °C during the day and 19-22 °C at night, in line with the low elevations at which these anurans live. The diet in captivity mostly consists of small hexapods such as *Drosophila melanogaster, D. hydei,* Collembola*, Thermobia domestica, Megoura viciae, Callosobruchus maculatus,*

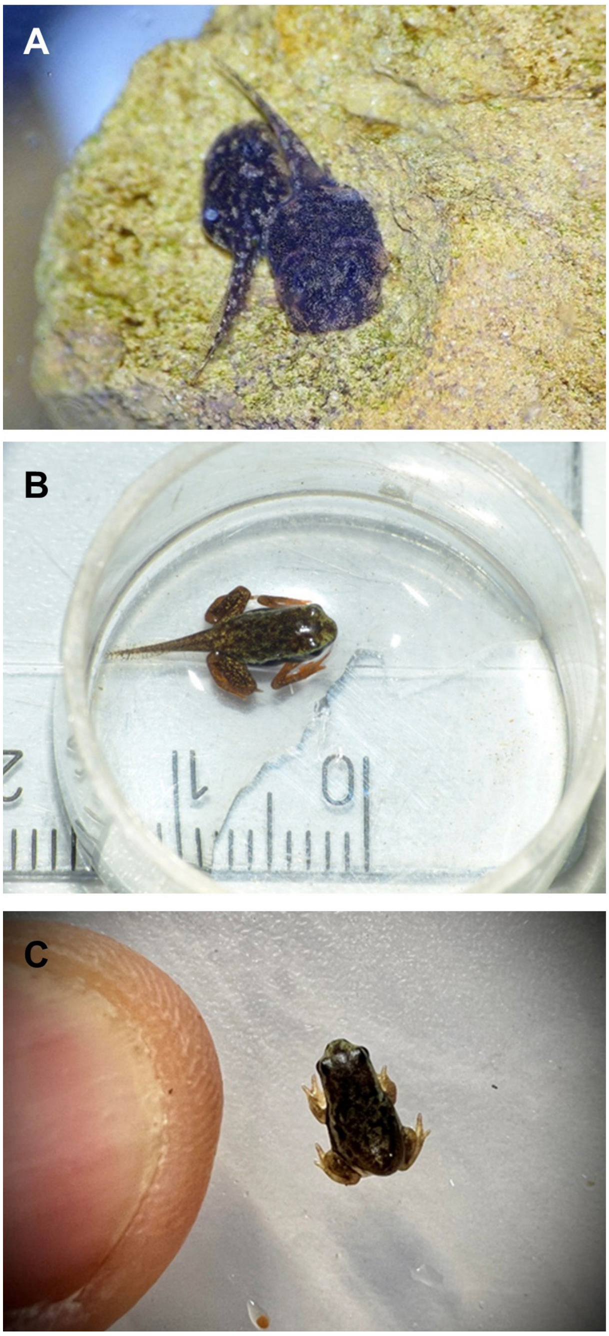


**Figure 4**: *Atelopus balios.* A) tadpoles; B) individual with not fully resorbed tail; C) neo-metamorphosed (© Thomas Ackermann)

and *Acheta domesticus* nymphs and a vitamin supplement to ensure complete nutrition, which is extremely important for their overall health (Buttermore et al., 2024).
When the female toads' bellies are swollen with eggs, they tolerate the males' attempt for amplexus and are put in a special aqua-terrarium simulating a gently flowing stream with oxygenated water. Spawning tanks have


**Figure 5**: A) Typical set-up for juveniles of *Atelopus balios;* B) Naturalistic-style terrarium for adults. (© Thomas Ackermann)

a water level of 4-15 centimetres, lots of rocks, bubbling stones and a pump with a moderate flow to ensure good aeration. The water is not heated and has a neutral pH with low water hardness. The females usually lay their eggs under rocks and other structures in the tank. Amplexus can extend for several days, with oviposition lasting up to nine hours, during which time the pair periodically emerge from the water to breathe (Fig. 3C). Each clutch consists of strings of several hundred unpigmented eggs, which hatch after around ten days (Fig. 3D) (Agustine et al., 2024). From hatching onwards, the tadpoles (Fig. 4A) attach themselves to any available structure in the aquarium, thanks to the anatomy of their buccopharyngeal area and the presence of a gastromyzophorous organ on their abdomen (Coloma & Lötters, 1996). This morphological feature is also present in the tadpoles of other *Atelopus* species. The morphology of the tadpoles is an adaptation to living in fast-flowing waters (dos Santos Dias & Anganoy-Criollo, 2024). The tadpole aquarium setup, which includes rocks, aeration stones and moving pumps, is not much different from the breeding aqua-terrarium of the adults. The tadpoles (Fig. 4A) take between 90 and 150 days (three to five months, depending on temperature and food supply) to leave the water, at which point they start feeding on springtails, mites and other tiny invertebrates once their tails have fully disapperead (Fig. 4B–C). The tiny froglets should be housed in plastic boxes containing wet clay and foliage. Artificial plants offer cover and shelter, and misting multiple times per day ensures proper hydration (Fig. 5A, B).

## Other *Atelopus* species in ex situ and reintroduction programs

While *Atelopus balios* provides an excellent model for general harlequin toad husbandry and captive breeding, it is one of many species included in such ex situ conservation programs. Despite successes in captive propagation, the permanent reintroduction of captive-bred offspring into their native habitats remains a significant and complex challenge. However, preliminary soft-release trials have been conducted for a few species to study the crucial transition from captivity to the wild. For instance, a release trial of the Critically Endangered *Atelopus varius* in Panama involved 458 captive-bred individuals to assess eco-ethological aspects, including dispersal and threat exposure (Klocke et al., 2023). This trial highlighted the difficulties in post-release monitoring: the frogs dispersed rapidly and were difficult to re-encounter. Effective tracking was achieved via radio transmitters applied to a small subset of individuals; even then, only half of the 30 radio-tagged individuals were trackable after 10 days, and no frog was sighted after 36 days post-release. The low re-encounter rates thus prevented insight into the animals' long-term fate. Crucially, the captive-bred individuals did not regain detectable levels of tetrodotoxin (TTX), the species' primary skin toxin, during the observation period.

Such acclimation and soft-release efforts often utilize mesocosms, cage-like enclosures placed within the species' original distribution area. These structures are designed to mimic the natural habitat, allowing the anurans to forage on naturally occurring invertebrates without artificial feeding. This same technique was employed for *A. limosus* (Estrada et al., 2022). After 27 days in the field mesocosms, the frogs' skin bacterial communities became comparable to those of wild conspecifics, supporting the hypothesis that the natural environment helps restore the skin microbiome. In terms of body condition, the overall average weight remained stable, consistent with the minimal change observed in females (a slight, non-significant loss) and the weight gain recorded in males. However, similar to *A. varius*, the individuals placed in mesocosms did not acquire the natural toxins. These trials collectively confirm the value of mesocosms for systematic monitoring of physiological and microbial changes during the critical transition period, even though the challenge of toxicity recovery persists.

### Conclusions

Ex situ conservation involves maintaining plants and animals outside their native habitat. This approach is primarily adopted when species are facing severe threats and the collection, rearing and breeding of individuals is the only means of preserving their evolutionary history. In the case of amphibians, for example, captive breeding not only safeguards many species from imminent extinction, as illustrated by the case of the *Atelopus* genus, but also complements in situ efforts. In addition to preventing population collapse, ex situ initiatives often serve as repositories of genetic diversity, ensuring that unique lineages are not lost even when wild populations decline dramatically.

Habitat restoration and the reintroduction of captive-bred offspring can significantly contribute to the stability of trophic networks, allowing species to recover ecological roles that might otherwise disappear. Programmes such as the CC, which is now active in Italy, involve experienced private breeders across Europe and demonstrate how integrating ex situ husbandry with field-based studies provides a sustainable model for long-term preservation. These collaborative frameworks also promote the exchange of knowledge between institutions, researchers and citizen scientists, fostering a broader awareness of the ecological importance of species protection.

Extending these practices to a wider range of species and advancing husbandry science will be essential to strengthening global conservation strategies, particularly for amphibians but ultimately for biodiversity as a whole (Conde et al., 2021; Bertram & Vivier, 2020). Furthermore, integrating ex situ and in situ efforts is critical in the context of climate change, where adaptive management is necessary (Byers et al., 2013). Looking ahead, the success of conservation programmes will increasingly depend on a combination of technological innovation, such as cryopreservation and genomic monitoring, and the capacity to adapt management practices to rapidly changing environmental conditions.

**Author Contributions**:

All authors (P.M.B., T.A., F.M.G.; E.B., F.A.) have equally contributed to the realization of this manuscript. The authors have read and agreed to the published version of the manuscript

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